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LITTON BIONETICS INC		
Document Title		
MUTAGENICITY EVALUATION OF 10558-10B IN THE AMES SALMONELLA/MICROSOME PLATE TEST (FINAL REPORT) WITH COVER LETTER DATED 011691		
Chemical Category		
TOLUENE DIISOCYANATE (26471-62-5)		

GRACE

86-910000640

CONTAINS NO CBI

Joseph W. Raksis, Vice President
Research Division

W.R. Grace & Co.-Conn.
7379 Route 32
Columbia, Maryland 21044
(301) 531-4331

January 16, 1991

91 JAN 24 AM 9:44

Environmental Protection Agency
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Attn: Health and Safety Reporting Rule (Notification/Reporting)

Please find attached 8(d) health and safety reports for mixtures processed containing toluene diisocyanate (CAS #26471-62-5), 4,4-Diphenylmethane diisocyanate (CAS #101-68-8) and 1,6-Diisocyanatohexane (CAS #822-06-0). Grace is submitting these reports for late filing since their submittal may have been subject to the isocyanates 10-year call-in of June 1, 1987.

We have reason to believe that some of these reports may have previously been submitted to EPA as attachments to PMN submissions. However, Grace is filing them as a precautionary measure to insure EPA's receipt.

These reports are being submitted for:

W. R. Grace & Co.-Conn.
Washington Research Center
7379 Route 32
Columbia, MD 21044

Sincerely,

J. W. Raksis
J. W. Raksis

A:\JR91-013/lw

Attachments - 20



86910000638

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FHP 2001 liquid
Lot 558
(Re-check)

Toluene Diisocyanate
26471-62-5
1,6 Diisocyanatoheptane
822-06-0

MUTAGENICITY EVALUATION OF

10558-10B

IN THE
AMES SALMONELLA/MICROSOME
PLATE TEST

FINAL REPORT

SUBMITTED TO:

W.R. GRACE AND COMPANY
7379 ROUTE 32
COLUMBIA, MARYLAND 21044

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20838

JULY 1978



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86910000640

- I. SPONSOR: W.R. Grace and Company
- II. MATERIAL*
 - A. Identification: 10558-108
 - B. Date Received: April 19, 1978
 - C. Physical Description: Viscous yellow liquid
- III. TYPE OF ASSAY: Ames Salmonella/Microsome Plate Test
- IV. PROTOCOL NO.: DMT-100
- V. RESULTS

The results of this assay are presented in Table 1.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats.

The compound was tested over a series of concentrations such that there was evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 1.0 μ l to 10 μ l per plate.

The compound was toxic to all the strains at 5 and 10 μ l per plate.

The results of the tests conducted on the compound in the absence of a metabolic activation system were all negative.

The results of the tests conducted on the compound in the presence of a rat liver activation system were positive with the strains TA-1538 and TA-98. The repeat tests performed with TA-98, also confirmed the initial positive results.

*Information was supplied by the sponsor. If information was not indicated by the sponsor, N.I. was entered.



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VI. INTERPRETATION OF RESULTS AND CONCLUSIONS (Continued)

The test compound, 10558-10B, exhibited genetic activity with the strains TA-1538 and TA-98, in activation assays conducted in this evaluation and is considered as mutagenic under these test conditions.

Submitted by:

Study Director

D. R. Jagannath

7.10.78

D. R. Jagannath, Ph.D.
Section Chief
Submammalian Genetics
Department of Genetics
and Cell Biology

Date

Reviewed by:

David Brusick

7/10/78

David Brusick, Ph.D.
Director
Department of Genetics
and Cell Biology

Date



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V. RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 10558-108

B. SOLVENT: DMSO

C. TEST INITIATION DATE: JUN. 9, 1978

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE											
			TA-1535		TA-1537		TA-1538		TA-98		TA-100		D4*	
			1	2	1	2	1	2	1	2	1	2	1	2
NONACTIVATION														
SOLVENT CONTROL	---	---	14		11		18		59		208		23	
POSITIVE CONTROL**	---	---	2022		504		1991		1491		1512		420	
TEST COMPOUND														
0.010000 UL	---	---	12		5		11		57		79		23	
0.100000 UL	---	---	5		4		10		54		89		13	
1.000000 UL	---	---	1		0		0		12		50		0	
5.000000 UL	---	---	0		0		0		0		0		0	
10.000000 UL	---	---	0		0		0		0		0		0	
ACTIVATION														
SOLVENT CONTROL	RAT	LIVER	15		10		18		36	38	127		25	
POSITIVE CONTROL***	RAT	LIVER	142		295		1426		1161	1450	1766		394	
TEST COMPOUND														
0.010000 UL	RAT	LIVER	12		10		17		45	--	143		20	
0.100000 UL	RAT	LIVER	11		15		30		52	--	115		19	
1.000000 UL	RAT	LIVER	21		12		34		82	108	142		30	
5.000000 UL	RAT	LIVER	14		19		71		69	110	128		15	
10.000000 UL	RAT	LIVER	12		14		72		113	111	104		5	

* 1RY+ CONVERTANTS PER PLATE

** TA-1535 ETHYLMETHANE SULFONATE 10 UL/PLATE
 TA-1537 QUINACRINE MUSTARD 10 UG/PLATE
 TA-1538 2-NITROFLUORINE 10 UG/PLATE
 TA-98 2-NITROFLUORINE 10 UG/PLATE
 TA-100 ETHYLMETHANE SULFONATE 10 UL/PLATE
 D4 ETHYLMETHANE SULFONATE 10 UL/PLATE

*** TA-1535 2-ANTHRAMINE 2.5 UG/PLATE
 TA-1537 2-ANTHRAMINE 2.5 UG/PLATE
 TA-1538 2-ANTHRAMINE 2.5 UG/PLATE
 TA-98 2-ANTHRAMINE 2.5 UG/PLATE
 TA-100 2-ANTHRAMINE 2.5 UG/PLATE
 D4 2-ANTHRAMINE 2.5 UG/PLATE

SOLVENT DMSO 50 UL/PLATE

SOLVENT DMSO 50 UL/PLATE

* THERE IS NO KNOWN POSITIVE CONTROL COMPOUND THAT WORKS
 WITH THIS STRAIN IN THE ACTIVATION PLATE ASSAYS.

PROTOCOL

1. PURPOSE

The purpose of this study was to evaluate the test material for genetic activity in a microbial assay with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Indicator Microorganisms

A description of strain verification is given in Standard Operating Procedure on page 10.

<u>Salmonella typhimurium</u>	TA-1535
	TA-1537
	TA-1538
	TA-98
	TA-100

<u>Saccharomyces cerevisiae</u>	D4
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B. Activation System

1. Reaction mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN (sodium salt)	4 μ mol
Glucose-6-phosphate	5 μ mol
Sodium phosphate (dibasic)	100 μ mol
MgCl ₂	8 μ mol
KCl	33 μ mol
Homogenate S9 fraction	0.1 \pm .05 ml

2. S9 homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill according to the procedure of Ames et al. (1975). S9 samples were coded by lot number and assayed for milligrams protein per milliliter and relative P448/P450 activity by methods described in LBI Technical Data on Rat Liver S9 Product.



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2. MATERIALS (Continued)

C. Positive Control Chemicals

The chemicals used for positive controls in the nonactivation and activation assays are given in Table 1 of Section V. Results.

D. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in Table 1 of Section V. Results.

3. EXPERIMENTAL DESIGN

A. Plate Test (Agar Incorporation)*

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, at least 4 dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, at least 4 dose levels of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hr at 37°C and scored for the number of colonies growing on each plate. O4 yeast plates were incubated at 30°C (nonactivation) and 37°C (activation) for 3-5 days and then scored. The concentrations of all chemicals are given in Table 1 of Section V. Results. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

* Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames agar incorporation method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.



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3. EXPERIMENTAL DESIGN (Continued)

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants (or convertants for D4) per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

4. EVALUATION CRITERIA

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over 2 or 3 log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the



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4. EVALUATION CRITERIA (Continued)

B. Dose-Response Phenomena

selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and 2-3 times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.



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4. EVALUATION CRITERIA (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g., TA-1537, responds to a mutagen in nonactivation tests, it will generally do so in activation tests (The converse of this relationship is not expected.). While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plate Test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann et al. (1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluations and interpretation of the data presented in this report are based only on the demonstration, or lack, of mutagenic activity.



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REFERENCES

Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. 72, 5135-5139.



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5. STANDARD OPERATING PROCEDURE

All data will be entered in ink (no pencil).

All changes or corrections in entries will be made with a single line through the change, and an explanation for the change must be written.

All calculations (weights, dilutions, dose calculations, etc.) will be shown on data records.

All data entries will be dated and initialed.

All laboratory operations will be written out in standard protocol manuals. These manuals will be present in each laboratory area.

Deviations from any established protocol will be described and justified.

Data will be stored in bound form (notebooks or binders). These bound data books will be reviewed by the appropriate Section Heads.

Chemicals submitted for testing will have date of receipt and initials of entering person.

Lot numbers for all reference mutagens, solvent, or other materials used in assays will be recorded.

Animal orders, receipts, and identification will be recorded and maintained such that each animal can be traced to the supplier and shipment. All animals on study will be properly identified.

A copy of the final report plus all raw data and support documents will be permanently stored in the archival system of Litton Bionetics, Inc.

Current curricula vitae and job descriptions will be maintained on all personnel involved in the study.

Salmonella strains will be routinely checked for the his, uvrB, rfa, and pKM 101 phenotypes. Only appropriately screened stock cultures will be used in chemical evaluations.



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CERTIFICATE OF AUTHENTICITY

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